Microscopic Algal Food of *Littorina planaxis* Philippi and *Littorina scutulata* Gould

(Gastropoda : Prosobranchiata)

BY

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Littorina planaxis and *L. scutulata* are commonly observed inhabiting the granitic upper littoral and supralittoral areas generally devoid of macroscopic algae. This study was undertaken to determine what microscopic algal types the animals were eating in these seemingly bare areas and to get an idea of the effects of this feeding on algal growth.

The study is conveniently divided into two parts: 1. Identification; 2. Determination of standing crop of microscopic algae and the effect of the snails grazing upon it.

1. Identification

METHODS

Rock specimens were removed from areas inhabited by the animals (especially *Littorina planaxis*) in the 6 to 8 foot range above mean zero tide. Their surfaces, crevices, and cracks were scraped into sterile sea water and then plated on an agar culture medium.

The media used were of two basic types. Medium 1 was made up of 2% agar in sterile sea water plus 10^{-3} molar Na_2HPO_4 and 10^{-3} molar NH_4NO_3. A soil extract (see Pringsheim, 1946) was prepared by mixing crushed granite with dark soil in 1 to 1 proportions. To this mixture was added 2 parts sea water and the soil-granite and water were heated at 90° C for one hour. The mixture was then filtered and the brownish liquid filtrate was added to the culture medium, 1 part soil extract to 10 parts agar plus sea water.

Medium 2 was identical with medium 1 except that NH_4NO_3 was omitted and 10^{-6} molar Na_2MoO_4 added. This medium was used to encourage the growth of possible nitrogen fixing blue-green algae present.

In addition to the above media, two more were made, one using medium 1 with a salt concentration in the sea water of four times normal, and another using medium 1 with *Littorina planaxis* mucus and feces spread over the surface. Both were prepared to approximate more closely the natural habitat of the algae.

Many snail shells, especially those of *Littorina planaxis*, have a very green color and are often deeply eroded and pitted. In addition, snails are normally observed crawling on one another. It was thought that the green may be a source of microscopic algal food, and perhaps the cause of the erosion. Therefore, the outer layer of a green shell and a brown eroded shell were scraped and plated on medium 1.

Snails of both species were collected after high high water while moving, dissected, and their stomach contents plated on both media 1 and 2 to culture any undigested pieces of algae.

Lastly, since the animals are splashed regularly during high tides, some sea foam was collected and plated to identify possible algal food sources not living directly in or on the granite rocks.

The cultures were placed upside down in front of a north window which had been covered with tissue paper to prevent overheating from direct sunlight.

In addition to culturing, rock specimens, shells, and stomach contents were examined directly in the laboratory.

RESULTS

The first growth in culture consisted of bacteria, but after ten days diatoms were very conspicuous, especially in medium 1 culture from rock surfaces. After 15 days most of the algae to be described began to grow, and three were subcultured.

The alga giving the common green coloration to round rocks in tide pools and to granite surfaces above tide pools in the high intertidal was identified as the chlorophyte, *Spongomorpha coalita* (Collins, 1909). This form has the general appearance of the description given by Smith (1944), but is a much smaller, juvenile stage. It was found much higher than Smith states, growing well within the 6 to 8 foot range in small cracks in the rock surface and

on the surface itself. It was also grown in stomach content cultures of both species and in sea foam culture. The growth in the latter was probably from fragments of alga washed from the rock surfaces.

The green alga growing in the outer layers of the shell of *Littorina planaxis* was identified as *Endocladia testarum* (Kylin, 1935). Previously, this alga had been described in the United States on the east coast only, inhabiting dead mollusk shells (see Thivy, 1943). Growth of this alga was also obtained from stomach contents of both species. When the outer layers of eroded shells were dissolved with HCl and the underlying material scraped off and examined, the alga was very prevalent, especially the massive, spherical thallus of overlapping, fused filaments. Since *Endocladia testarum* grew well from scrapings of a brown eroded shell with no visible surface green, it may be the agent responsible for the shell erosion.

Prominent among the blue-green algae found were *Plectonema terebrans* (Bornet & Flahault, 1889), *Calothrix pilosa* (Harvey, 1858), and *Calothrix crustacea* (Bornet & Thuret, 1878). These algae fit the general description given by Umezaki (1961). *Plectonema terebrans* differs from Umezaki's description, being found here in shells of live *Littorina planaxis* and on the granite rocks in close association with the other blue-green algae and with *Spongomorpha coalita* previously mentioned. It could also be responsible for some of the erosion of shells, since it is a shell boring form. All three of these blue-green algae were found from cultured stomach contents, and *P. terebrans* was found in cultured sea foam (probably washed from rocks). *Calothrix pilosa* was very abundant in the cultured stomach contents of *L. planaxis*. Generally, the blue-green algae did as well in medium 2 as medium 1, so the presence of nitrogen fixing forms is possible.

Dermocarpa sp. (Crouan, 1858. See Smith, 1950) and *Spirulina* sp. (Turpin, 1892. See Umezaki, 1961), two other blue-green algae, were found in limited quantities. The *Spirulina* was found in association with *Plectonema terebrans* on rock surfaces, and *Dermocarpa* was found growing on *Rhodochorton Rothii* (Nageli, 1862) filaments in the field only.

Rhodochorton Rothii was the only red alga found, commonly in crevices receiving very little sunlight. *Littorina planaxis* is often observed in these crevices and some pieces of *R. Rothii* were found in their stomach contents, although none grew in culture, probably because of its unusually cold, damp habitat. This alga fits the description given by Smith (1944) except for its growing in crevices.

Diatoms and unicellular green and blue-green algae were found in most cultures but no attempt was made to identify them. The diatoms were especially abundant both in rock surface cultures and stomach content cultures of both species, and seem to constitute one of the primary food sources of the snails. (see Castenholz, 1961).

2. Determination of Standing Crop and *Littorina* Grazing Effects

METHODS

a. Chlorophyll Content: In an effort to determine the standing crop of all the microscopic algae in the 6 to 8 foot range, areas of rock were chipped from the surface with hammer and chisel. The chlorophyll was extracted with methyl alcohol and its absorption spectra determined. These spectra were compared with the absorptoin spectrum of a similar size piece of *Ulva*.

The rock samples extracted were 5 cm by 5 cm square and from 1 to 10 mm deep, depending on the depth of the green coloration beneath the surface. Because this depth varied, all calculations were based on surface area and not volume.

The rock samples were crushed with a mortar and pestle, placed in dark screw cap bottles, and covered with 50 ml absolute alcohol per 25cm^2 of rock surface. Acetone and ethyl alcohol were tried but did not effect complete extraction. The bottles were placed in a refrigerator for 29 hours, contents filtered through No. 1 filter paper, and immediately analyzed on a Beckman Model DUR spectrophotometer at wave-lengths from 430 to 680 millimicrons.

Samples of *Ulva* were cut into 1 cm squares and extracted in a similar manner without grinding. The values obtained were modified by multiplying by 25 to give values for 25 cm^2 *Ulva*/50 ml alcohol.

To estimate the algal content of the *Littorina planaxis* shells, snails were removed and their shells crushed and extracted as above. The absorption spectrum was corrected to 25 cm^2 shell surface / 50 ml alcohol. The shell surface area estimations are described under Photosynthetic Rate.

b. Photosynthetic Rate: Rock samples were removed intact from outcroppings and a 25 cm^2 surface area exposed, the rest of the rock covered with aluminum foil to prevent light from entering. The rocks were placed in one liter jars filled with sea water of a known O_2 concentration, and placed in the sun for ½ hour. The rocks were then removed and the water analyzed by the Winkler method to determine O_2 increase. From this, carbon production was calculated.

Ulva's photosynthetic rate was previously determined (see under Final Determination of Standing Crop).

The photosynthetic rate of the algae on the snail shells *(Littorina planaxis)* was determined by cracking the shells to remove the snails and putting the shells in sea water in direct sunlight. O_2 increase was again measured by the

Winkler method. Snail shell surface area was calculated by approximating with $1/\pi$ of $\pi R\sqrt{R^2+H^2}$, the formula for the area of the curved surface of a right circular cone. R here is the distance across the opercular opening and H the height of the shell from the bottom of the opercular opening to the top of the spire (see NORTH, 1954).

c. **Respiration Rate:** Only the respiration rate for the algae on a 25 cm^2 granite surface was measured. This was accomplished by chipping a rock to the desired dimensions and putting it in sea water in the dark for 12 hours. O_2 concentration before and after was measured by the Winkler method and O_2 decrease calculated.

RESULTS

a. **Chlorophyll Content:** The absorption spectra of methanol extracts from an average of three 25 cm^2 rock surfaces, three *Ulva* samples, and four crushed shells were determined. Since chlorophyll (a) is present in all the algae found, this was used as a standard of comparison.

Using Strickland and Parson's method (1960), chlorophyll (a) content was determined by the formula

$$Cla = 15.6\ E_{665} - 2.0\ E_{645} - 0.8\ E_{630}$$
$$= \text{mg chlorophyll (a) / liter of solvent}$$

This formula measures absorption with a 1 cm cell, with E being extinction or absorption. Values obtained from samples were first multiplied to give 25 cm^2 surface area and then multiplied by the number of milliliters used in extraction over 1000 to give Cl(a) present per liter of solvent. This figure was then multiplied by 400 to convert to m^2 of surface area.

The value for the algae on the granite surfaces in the 6 to 8 foot tidal range (av. of 3 samples):

$$= .043 \text{ g Cl (a) / } m^2$$

This figure is approximate because methanol does not extract the main pigments in the blue-green algae.

Ulva chlorophyll (a) content using the same formula:

$$= .081 \text{ g Cl(a) / } m^2 \text{ (av. of 3 samples)}$$

Shell chlorophyll (a) content was not calculated because after 29 hours the shells were not completely extracted. It is significant that even with incomplete extraction the shells had a higher pigment content than either the *Ulva* or the rock.

b. **Photosynthetic Rate:** Rock surfaces (av. of 4 tests):

$$= 7.8 \text{ ml } O_2/25 \text{ cm}^2/\text{day}$$
$$= 3120 \text{ ml } O_2/m^2/\text{day (assuming 12 hours of light in a day)}$$

Ulva previously determined for Hopkins Marine Station (see BLINKS, 1955):

$$= 3 - 7.2 \text{ g carbon / } m^2 \text{ / day}$$

Algae on shells *(Littorina planaxis):*

$$= .981 \text{ g carbon / } m^2 \text{ / day}$$

c. **Respiration Rate:** Algae on rock surfaces (av. of 3 tests):

$$= 246.8 \text{ ml } O_2 / m^2 / \text{day}$$

Final Determination of Standing Crop

Since the photosynthetic rate of the algae on the granite surfaces is about half that of *Ulva*'s minimum value, and the chlorophyll (a) content of the algae on the granite surfaces is approximately half that of an equal surface of *Ulva,* then it is assumed that chlorophyll (a) content and standing crop are related and that the relation for *Ulva* is proportional to that of the algae on the granite surfaces. If this assumption can be made, then dividing *Ulva*'s chlorophyll (a) content into that of the rock:

$$.043 \div .081 = .53$$

Standing crop of *Ulva* for Hopkins Marine Station (BLINKS, 1955).

$$= 70 \text{ g carbon / } m^2$$

Then the standing crop of algae on the granite surfaces

$$= (70)\ (.53) = 37 \text{ g carbon / } m^2$$

Dividing standing crop by production rate we get time for the algal crop on the rocks:

$$= 37 \div 1.66 = 22.3 \text{ days.}$$

A chart summarizing the results of standing crop determinations is presented in Table I.

Effects of Grazing

METHODS

For this determination a flat surface was selected at the 6 foot tide level. Six baskets of 15 cm by 15 cm by 5 cm size were fastened to the rock by bolts driven into the surface. The area received abundant splash and spray during high tides and was inhabited by snails of both species, but primarily *Littorina planaxis.*

Ten snails *(Littorina planaxis)* with a volume of .27 cc / snail were introduced into each of three of the baskets while the other three baskets were kept empty.

After 25 days the baskets and snails were removed and 10 cm^2 sections of granite were chipped from the center of each basket-covered area. The sections were analyzed as in previous chlorophyll (a) determinations to ascertain any differences in pigment content which could be used as a measure of algal food consumed.

RESULTS

The absorption spectra of extracts of grazed and ungrazed surfaces were determined, but since chlorophyll (a) content was not known before the test, its calculation would not be meaningful.

For a general measure, subtracting the absorption values for grazed and ungrazed surfaces at the peaks gave

TABLE I: Summary of Standing Crop Determinations

Type of Surface	Chlorophyll (a) content grams/m²	Respiration rate ml O_2/m²/day	Production and Crop: Photosynthetic rate grams carbon/m²/day	Production and Crop: Standing crop grams carbon/m²	Production and Crop: Time for crop days
Ulva	.081	-------	3 to 7.2	70	10 to 23
Algae on Granite Surfaces	.043	286.8	1.66	37	22.3
Algae on *Littorina planaxis* Shells	-------	-------	.981	-------	-------

a value of from 6% to 10%. Therefore, 10 snails with a volume of 2.7 cc grazing on a 225 cm² surface of granite reduced the pigment content by approximately 8% when compared with an ungrazed surface over a period of 25 days. This figure is only approximate, since a standard deviation cannot be calculated.

SUMMARY

1. The following algae were identified in the habitat of *Littorina planaxis* and *Littorina scutulata* (* denotes positive identification as food):

**Spongomorpha coalita*
**Endocladia testarum* (on shells)
**Plectonema terebrans*
**Calothrix pilosa*
**Calothrix crustacea*
**Rhodochorton Rothii*
Dermocarpa sp.
Spirulina sp.
*Unicellular green and blue-green algae
*Diatoms

2. The standing crop of microscopic algae on granite surfaces is about half that of *Ulva*. The pigment content of the algae on the shells of *Littorina planaxis* is much greater than that of a rock or *Ulva* surface of similar size.

3. In 25 days, ten snails reduce the algal pigment content of a 225 cm² granite surface by approximately 8% when compared with an ungrazed surface of similar size.

NOTES & NEWS

ERRATA

BY

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In "The Cypraeidae of Fiji," published in The Veliger 6 (4): 177 - 201, the following passages should be emended:

p. 180 - bottom, for: Synonyms are shown in brackets [ ] read: generic and subspecific terms are shown in brackets [ ]

p. 182 - No. 4, for: *nomen nudum* read: non binominal

p. 188 - No. 25, for: *Monetaria annulus sosokoana* LADD, read: *Cypraea (Monetaria) annulus sosokoana* LADD

p. 189, - No. 29, for: *Cypraea erosaria agassizi* LADD, read: *Cypraea (Erosaria) agassizi* LADD

p. 192: Explanations for figures 15 and 15 a belong to plate 23, not plate 24

p. 193 - No. 39, for: *Erronea cauricathema* IREDALE, read: *Erronea caurica thema* IREDALE

p. 193 - No. 41: the year 1938 should be placed after SCHILDER & SCHILDER

p. 194 - No. 44, for: *Cypraea lutea* GRONOW, 1781, read: *Cypraea lutea* GMELIN, 1791.

p. 198 - No. 55, for: *Bistolida stolida trakau* STEADMAN & COTTON, read: *Bistolida stolida thakau* STEADMAN & COTTON

p. 200: Opinion 261 of I.C.Z.N rejects Gronovius' Zoophylacium Gronovianum for nomenclatorial purposes. Gronovius' *Amphiperas* must be replaced by *Ovula* BRUGUIÈRE, 1789, and the heading Amphiperatidae should be changed to Ovulidae.

No. 2: *Ovulum angulosum* LAMARCK 1822, should be emended to *Ovula angulosa* LAMARCK 1822, as *Ovulum* was not established by LAMARCK, but by SOWERBY in 1828